

## Potential use of stable isotopes in amino sugars as tracers for soil organic matter translocations from critically degraded areas in farm lands

By S. Bodé and P. Boeckx<sup>1</sup>

Laboratory of Applied Physical Chemistry (ISOFYs), Faculty of Bioscience Engineering, Ghent University, Belgium,  
[pascal.boeckx@ugent.be](mailto:pascal.boeckx@ugent.be)

### Hypothesis and challenge

Soil erosion and subsequent sedimentation are natural processes caused by water, wind and ice. Anthropogenic activities such as deforestation, overgrazing, changes in land use, non-sustainable farming practices and global climate change tend to accelerate soil erosion. The result is degradation of the landscape which has an impact on soil fertility, crop productivity, water pollution, and sedimentation in lakes, reservoirs and floodplains. New methods are emerging to assess carbon storage and mobilization within a catchment in order to investigate the relationship between sites with critical soil erosion (source) and deposition areas (sink). Compound specific stable isotope analysis (CSSIA) is a promising approach to fingerprint soil organic matter (SOM) via its molecular components. Evidence is emerging that CSSIA of plant root derived fatty acids (Gibbs, 2008) also enables us to establish carbon source-sink relationships in catchments that are affected by considerable erosion and deposition processes.

To a certain extent, micro-organisms derive their building blocks from plant-root derived organic compounds (Denef et al. 2009). Cell walls of fungi, bacteria and actinomycetes are partially constructed from polymers of acetylated amino sugars. Because of the relative stability of these compounds after cell death, amino sugars have been used to quantify the relative contribution of the different functional groups to living and dead soil microbial communities, which in turn can be used to explain land use changes. We therefore hypothesize that the relative abundance of the different amino sugars, together with their isotopic composition ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) is characteristic of a particular land use and management practice. For these reasons CSSIA of amino sugars could become a tool to track and quantify soil erosion hotspots. Methods to determine the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of amino sugars using gas chromatography/combustion/isotope ratio mass spectrometry (GC-c-IRMS) have been proposed in the literature. However, due to the laborious method used for sample preparation (derivatisation), the likelihood that the obtained  $\delta^{13}\text{C}$  values are uncertain, is too high for this method to be used for natural abundance studies (Decock et al. 2009). Therefore, the first challenge was to develop and validate a high-performance liquid chromatography isotope ratio mass spectrometry (HPLC-c-IRMS) methodology with improved accuracy and precision.

### Method development

The amino sugars extraction procedure was based on the method described by Zhang and Amelung (1996). In short dry and 2mm sieved soil was hydrolysed with 6M HCl for 8 hours at 105°C. The filtrate was then dried at 45°C at reduced pressure before being re-dissolved and applied on cation exchange resin in  $\text{H}^+$  form. The neutral and negatively charged compounds were then washed out with water. The amino sugars were eluted with 12 mL of 0.5 M HCl. The eluate was dried again under reduced pressure, re-dissolved in 1 mL of  $\text{H}_2\text{O}$ , dried and kept refrigerated until analysis. The chromatography separation was performed on an anion exchange column using 2mM NaOH and a column temperature of 15°C to elute the basic amino sugars (glucosamine, mannosamine and galactosamine), while for muramic acid 2mM  $\text{NaNO}_3$  was added to the 2mM NaOH and the column was set at 30°C (see Bodé et al. 2009 for details).

### Evaluation

Reference amino sugar solutions were analyzed with the two developed methods. Different amounts of the basic amino sugars glucosamine and galactosamine were injected (ranging from 0.5 to 100 nmol) and the isotopic composition and peak areas of the individual amino sugars were determined. The  $\delta^{13}\text{C}$  obtained for the individual amino sugars was compared to the isotopic composition of the pure reference material as determined by  $\mu\text{EA}^2$ -IRMS analysis (Figure 1b). The repeatability and obtained  $\delta^{13}\text{C}$  values were not significantly concentration dependent as long as the injected amount was higher than 1.5 nmol. Beneath this amount the repeatability decreased rapidly (Figure 1a).

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<sup>2</sup> Micro element analysis

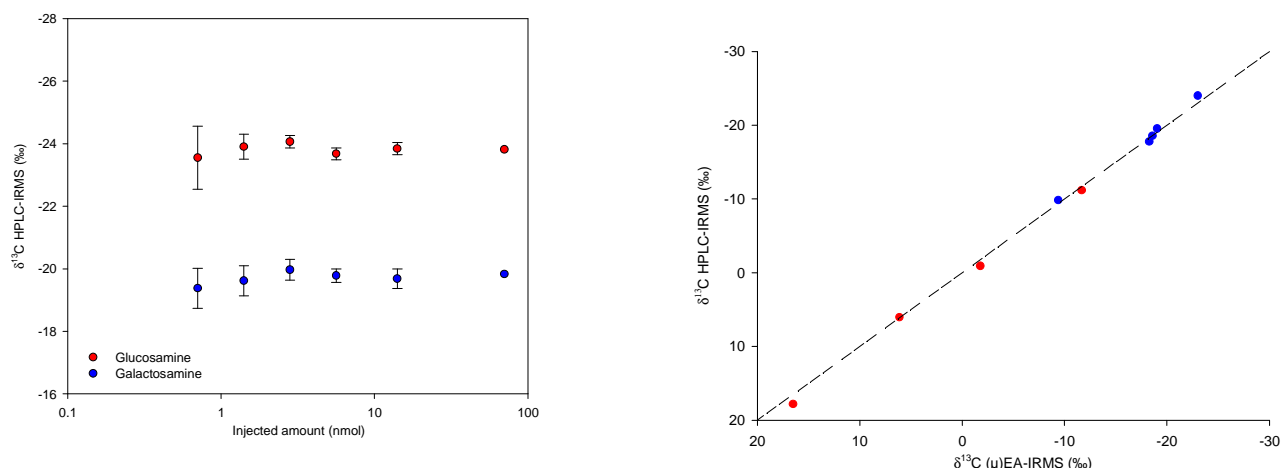


Figure 1. (a) Determination of the isotopic composition of reference amino sugars in function of injected amount (error bars represent plus minus one standard deviation). (b)  $\delta^{13}\text{C}$  as determined by HPLC-c-IRMS compared to  $\delta^{13}\text{C}$  determined by  $\mu\text{EAIRMS}$ . The blue dots are the reference amino sugars (Glucosamine, galactosamine, mannosamine and muramic acid) at natural abundance; the red dots are  $^{13}\text{C}$  enriched glucosamine solutions; the full line is the 1:1 line

To assess the recovery of the hydrolysis step and the accuracy of the  $\delta^{13}\text{C}$  determination of soil amino sugars, soil samples were spiked with different amounts of glucosamine, galactosamine and muramic acid. The recovery for Glucosamine, galactosamine and muramic acid were 72, 91 and 78 % respectively (Table 1). The isotopic composition of the amino sugars in these spiked samples was measured and compared to its theoretical isotopic composition.

$$\delta^{13}\text{C}_{\text{theo}} = \frac{N_{\text{soilAS}} \cdot \delta^{13}\text{C}_{\text{soilAS}} + N_{\text{spike}} \cdot \delta^{13}\text{C}_{\text{spike}}}{N_{\text{soilAS}} + N_{\text{spike}}}$$

Table 1.  $\delta^{13}\text{C}$  (‰) and concentration of amino sugar in a soil extract as measured by HPLC-c-IRMS. The recovery and deviation in absolute values of the calculated  $\delta^{13}\text{C}$  values of soils spiked with glucosamine, galactosamine and muramic acid is also presented.

	Soil extract (n=4) concentration <sup>1</sup> (mg kg <sup>-1</sup> soil)	HPLCIRMS	extraction of spiked Soil (n=2) recovery (%)	$ \Delta\delta^{13}\text{C} $ <sup>2</sup> (‰)
Glucosamine	885 (± 14)	-26.3 (±0.2)	72 (±5)	0.3 (± 0.4)
Galactosamine	299 (± 13)	-25.2 (±0.1)	91 (±13)	0.3 (± 0.3)
Muramic acid	26 (± 2)	< LoQ	78 (±5)	0.7 (±0.2)

1 The recovery of xylose was used to correct losses during purification steps

2  $\Delta\delta^{13}\text{C}$  (‰) is the difference between the measured  $\delta^{13}\text{C}$  of soil samples spiked with 20, 50 and 100  $\mu\text{g}$  reference standards and the theoretical ( $\delta^{13}\text{C}_{\text{theo}}$ ) calculated by summation of the relative contribution of  $\delta^{13}\text{C}$  from soil native amino sugars and  $\delta^{13}\text{C}$  from amino sugars used to spike the soil.

## Conclusions

A HPLC-c-IRMS methodology, to determine  $\delta^{13}\text{C}$  and the concentration of individual amino sugars present in soil, excluding a derivatisation step, has been successfully developed. This newly developed method could prove to be very useful in several research areas including dynamics of amino sugars in soils and these compounds could be used as fingerprints to pinpoint the origin of sediments in a catchment. The method provided reproducible, accurate and precise measurements for the determination of  $\delta^{13}\text{C}$  of amino sugars as well for their quantification. The next step will be to assess whether different land uses (deciduous vs. pine forest, extensive vs. intensively managed grassland, and conventionally tilled vs. no-till arable land) can be differentiated via  $\delta^{13}\text{C}$  (HPLC-c-IRMS) and  $\delta^{15}\text{N}$  (GC-c-IRMS).

## References

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## Combined use of $^{137}\text{Cs}$ and stable isotopes to assess soil degradation in mountainous grasslands of Switzerland

By K. Meusburger<sup>1</sup>, M. Schaub<sup>1</sup>, N. Konz<sup>1</sup>, L. Mabit<sup>2</sup> and C. Alewell<sup>1</sup>

<sup>1</sup> Department of Environmental Sciences, Institute of Environmental Geosciences, Basel University (Switzerland)

<sup>2</sup> Soil and Water Management & Crop Nutrition Subprogramme, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency (IAEA), Vienna - Seibersdorf, Austria

### The Challenge

Current concerns to ensure a sustainable use and management of European soil and agricultural water resources generate an urgent need to obtain reliable quantitative data on the extent and actual magnitude of soil erosion. Soil loss and its triggering processes are still subject to a high degree of uncertainty in European mountainous areas. Investigations to describe and predict soil erosion in such areas of reduced accessibility are urgently needed to improve quantification and process understanding (Asselman *et al.*, 2003; Alewell *et al.*, 2008). This multi-isotopic approach aims to assess soil loss and the quantitative relevance of different erosion processes in a sub-alpine catchment of the Central Swiss Alps (Urseren Valley).

### Experimental design

The approach used in the present study combines direct measurement of sediment yield during the growing season of 2007 with long-term soil erosion assessment based on in-situ and laboratory fallout radionuclide (i.e.  $^{137}\text{Cs}$ ), and stable carbon ( $^{13}\text{C}$ ) and oxygen isotope ( $^{18}\text{O}$ ) measurements in agricultural lands. Remote sensing was also used to up-scale the obtained information.

### Main results

The comparison of the different soil erosion assessment techniques from nine slopes resulted in large discrepancies. Erosion rates measured with sediment traps during the growing season were below  $1.5 \text{ t ha}^{-1} \text{ a}^{-1}$  (Konz *et al.*, 2009). However,  $^{137}\text{Cs}$  based erosion rates indicated high erosion rates of up to  $37 \text{ t ha}^{-1} \text{ a}^{-1}$ , which were in agreement with the significant visible erosion damage observed at the study sites (Figure 1; Konz *et al.*, 2009). The high erosion rates revealed by the  $^{137}\text{Cs}$  technique can be explained by the fact that  $^{137}\text{Cs}$  measurements integrate a longer soil erosion time period from April 1986 (Chernobyl being the main source of  $^{137}\text{Cs}$  fallout in this area) until today, including erosive processes during the winter season and snow melt in spring, as well as during intensive rain storm events in summer. Thus, in the Urseren Valley, soil erosion processes during the growing season seemed to have only a minor influence on the mean annual soil erosion rates, if no extreme and exceptional events occurred. The results highlighted that winter processes, especially mechanical translocation by snow movement (gliding, ablation and avalanches), are of major importance (Figure 2). Inventories of  $^{137}\text{Cs}$  on grasslands with differing fractional vegetation cover<sup>3</sup> showed significant

<sup>3</sup> Fractional vegetation cover is a dynamic variable which changes on a daily basis, yet to this point most models simply include a climatological value for the percent healthy vegetation cover. Remote sensing from satellites now provides enough detailed and continuous coverage of the Earth's surface to provide an indirect, NDVI-based vegetation cover product.